

RESEARCH ARTICLE

Testing the Mantel statistic with a spatially-constrained permutation procedure

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Abstract

1. Mantel tests are widely used in ecology to assess the significance of the relationship between two distance matrices computed between pairs of samples. However, recent studies demonstrated that the presence of spatial autocorrelation in both distance matrices induced inflations of parameter estimates and type I error rates. These results also hold for partial Mantel test which is supposed to control for the spatial structures.
2. To address the issue of spatial autocorrelation in testing the Mantel statistic, we developed a new procedure based on spatially constrained randomizations using Moran spectral randomization. A simulation study was conducted to assess the performance of this new procedure. Different scenarios were considered by manipulating the number of variables, the number of samples, the regularity of the sampling design and the level of spatial autocorrelation.
3. As identified by previous studies, we found that Mantel statistic and its associated type I error rate are inflated in simple and partial Mantel tests when both distances matrices are spatially structured. We showed that these biases increased with the number of variables, decreased with the number of samples and were slightly lower for regular than irregular sampling. The new procedure succeeded in correcting the spurious inflations of the parameter estimates and type I error rates in any of the presented scenarios.
4. Our results suggest that studies from several fields (e.g. genetic or community ecology) could have been overestimating the relationship between two distances matrices when both presented spatial autocorrelation. We proposed an alternative solution applicable in every field to correctly compute Mantel statistic with a fair type I error rate.

KEYWORDS

distance decay relationships, Mantel test, Moran spectral randomization, principal coordinates analysis, spatial autocorrelation, spatially constrained null model, type I error inflation

1 | INTRODUCTION

The Mantel test is a well-known statistical procedure pertaining to the distance decay relationships framework (Nekola & White, 1999)

which assesses the correlation between two distance matrices computed between pairs of samples and evaluates its significance using random permutations (Mantel, 1967). Although criticized in the literature concerning its assumption of a linear relationship between the two distance matrices, the formulation of its null hypothesis and the interpretation of its statistic which are not as trivial as with raw data

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(Legendre, Fortin, & Borcard, 2015), this test is still widely used by ecologists from different fields. For instance in community ecology, Mantel test is often used to disentangle the roles of habitat selection and dispersal processes on community and metacommunity organization (Jones, Tuomisto, Clark, & Olivas, 2006; Moritz et al., 2013; dos Santos, Saraiva, Müller, & Overbeck, 2015). In molecular ecology, Mantel test is routinely used to test the link between two matrices of phenotypic (resp. genetic) distances measured on individuals or populations (Richardson, Brady, Wang, & Spear, 2016; Shafer & Wolf, 2013; Storfer, Murphy, Spear, Holderegger, & Waits, 2010).

When distance matrices are computed on samples located in space, a major problem lies in the possible presence of spatial autocorrelation (Legendre & Fortin, 2010; Meirmans, 2012). Spatial autocorrelation is a well-known problem in statistical ecology (Sokal & Oden, 1978) as it violates the assumption of data independence required in many statistical methods (Diniz-Filho, Bini, & Hawkins, 2003; Legendre & Legendre, 1998). As such, it induces an inflation of type I error rate; that is, rejecting the null hypothesis too often (Cliff & Ord, 1981). Mantel tests have been shown to be strongly affected by spatial autocorrelation when present in both distance matrices (Guillot & Rousset, 2013; Legendre et al., 2015; Oden & Sokal, 1992). As such, the partial Mantel test was developed (Smouse, Long, & Sokal, 1986) to control for spatial structures when testing the link between the two distance matrices of interest.

However, through a deep investigation of simple and partial Mantel tests, Guillot and Rousset (2013) showed that partial Mantel test was unable to correct for the effect of spatial autocorrelation and that both tests presented inflated type I error rates. The problem lies in the random permutation procedure which breaks the potential dependencies between distance matrices (as expected by the null hypothesis) but also their inherent autocorrelation structures (Guillot & Rousset, 2013). To solve this issue, Guillot and Rousset (2013) mentioned different alternatives including shift permutations (Upton & Fingleton, 1985). This type of permutation allows randomizing the data to break the link between the two distance matrices, while preserving their individual spatial structures so that they are taken into account in the testing procedure. Nevertheless, shift permutations can only be applied when samples originate from a regular grid, whereas many empirical studies implement irregular samplings for practical reasons. Following this idea, we propose here another strategy using Moran spectral randomization (MSR; Wagner & Dray, 2015). This spatially constrained randomization procedure initially developed in the simple case of bivariate correlation allows generating random replicates that preserve the original spatial structures of the data while breaking their correlations. Contrary to shift permutations, this procedure can be applied to regular or irregular samplings.

In this study, we first performed a simulation study to illustrate how simple and partial Mantel tests can be affected by spatial autocorrelation. Second, we proposed and evaluated a new approach based on MSR to improve the testing and computing of the Mantel statistic in the presence of spatial autocorrelation.

2 | MATERIALS AND METHODS

2.1 | Simple and partial Mantel tests

The Mantel test considers two $n \times n$ symmetric matrices \mathbf{D}_X and \mathbf{D}_Y containing pairwise distances among n samples. If original data consist in raw data stored in tables \mathbf{X} and \mathbf{Y} (i.e. samples by variables), they should be transformed in distance matrices \mathbf{D}_X and \mathbf{D}_Y prior to the computation of the Mantel statistic.

The observed Mantel statistic (r_{M-obs}) is defined as the sum of cross products between both distance matrices \mathbf{D}_X and \mathbf{D}_Y :

$$r_{M-obs} = \sum_{i=1}^{n-1} \sum_{j=i+1}^n \mathbf{D}_{X_{ij}} \mathbf{D}_{Y_{ij}}. \quad (1)$$

Partial Mantel test, introduced by Smouse et al. (1986), is widely used in ecology to control for spatial autocorrelation present in the data. The test considers \mathbf{D}_X , \mathbf{D}_Y and an additional distance matrix \mathbf{D}_Z which can be derived from a raw data table \mathbf{Z} . To control for spatial structure, \mathbf{Z} generally contains geographical coordinates so that \mathbf{D}_Z represents the geographical distances between samples. Partial Mantel test is based on the correlation coefficient between \mathbf{D}_X and \mathbf{D}_Y while controlling for the effect of the third matrix \mathbf{D}_Z .

The significance of simple and partial Mantel tests is assessed with a permutation procedure. The two matrices (\mathbf{D}_X and \mathbf{D}_Y , or equivalently \mathbf{X} and \mathbf{Y} if \mathbf{D}_X and \mathbf{D}_Y are obtained from raw data) are permuted independently (i.e. rows and columns are permuted in the same manner for distance matrices; only rows for raw data) and the Mantel statistic is recomputed. This permutation procedure is repeated n_{RND} times (e.g. $n_{RND} = 999$) to obtain the distribution of the Mantel statistic under the null hypothesis. Note that permuting both matrices is not required as this distribution can be obtained by permuting only one matrix (\mathbf{D}_X or \mathbf{D}_Y). The observed value of the statistic r_{M-obs} is then compared to this distribution to assess its significance. The null hypothesis H_0 associated with this testing procedure states that “the distances in \mathbf{D}_X are not linearly related to the corresponding distances in \mathbf{D}_Y ” (Legendre et al., 2015).

2.2 | Overcoming the spatial autocorrelation problem with an alternative randomization procedure

As used in Mantel tests, classical permutation procedures assume implicitly that samples are exchangeable. The presence of spatial autocorrelation induces a kind of pseudo-replication (Hurlbert, 1984) leading to a violation of the exchangeability assumption hence an inflation of type I error rates and imprecise parameter estimates. To address the issue of spatial autocorrelation in testing the Mantel statistic, we used the MSR (Wagner & Dray, 2015) instead of standard permutation procedure. The MSR aims at producing random replicates which preserves the spatial structures of the original variables so that spatial autocorrelation is taken into account in the testing procedure.

Moran spectral randomization starts by defining a $n \times n$ spatial weighting matrix \mathbf{W} . This matrix is a mathematical representation of the geographical layout of the region under study. The spatial weights

reflect a priori the absence ($w_{ij} = 0$), presence or intensity ($w_{ij} > 0$) of the spatial relationships between the samples i and j . The doubly centred matrix \mathbf{W} is diagonalized and we define $\Lambda = \text{diag}(\lambda_1, \dots, \lambda_{n-1})$ the diagonal matrix of eigenvalues and \mathbf{V} the $n \times (n-1)$ matrix with associated eigenvectors $\mathbf{v}_1, \dots, \mathbf{v}_{n-1}$ stored as columns. These eigenvectors, named Moran's Eigenvector Maps (MEM) by Dray, Legendre, and Peres-Neto (2006), are orthogonal and maximize spatial autocorrelation measured by the Moran's index of spatial autocorrelation. If we consider a centred variable \mathbf{x} , the Moran's index of autocorrelation is equal to (Dray, 2011):

$$I(\mathbf{x}) = \sum_{i=1}^{n-1} \lambda_i \text{cor}^2(\mathbf{v}_i, \mathbf{x}) \quad (2)$$

with

$$\sum_{i=1}^{n-1} \text{cor}^2(\mathbf{v}_i, \mathbf{x}) = 1 \quad (3)$$

The variable \mathbf{x} can thus be entirely decomposed on the orthogonal basis of MEM as follows:

$$\mathbf{x} = \sum_{i=1}^{n-1} \mathbf{v}_i \text{cor}(\mathbf{v}_i, \mathbf{x}) \quad (4)$$

This decomposition allows to define a scalogram (Dray et al., 2012), depicting the multiscale structure of \mathbf{x} , where each MEM explains a proportion of the variance of \mathbf{x} equal to $\text{cor}^2(\mathbf{v}_i, \mathbf{x})$. In its strictest version, the MSR algorithm aims to find a set of coefficients a_1, \dots, a_{n-1} to define a new variable $\mathbf{x}_{\text{MSR}} = \sum_{i=1}^{n-1} a_i \mathbf{v}_i$ with the following additional constraints:

$$I(\mathbf{x}) = I(\mathbf{x}_{\text{MSR}}) = \sum_{i=1}^{n-1} \lambda_i a_i^2 \quad (5)$$

$$a_i^2 = \text{cor}^2(\mathbf{v}_i, \mathbf{x}) \quad (6)$$

$$\sum_{i=1}^{n-1} a_i^2 = 1 \quad (7)$$

This ensures that the new variable \mathbf{x}_{MSR} (MSR replicate) has the same global level of spatial autocorrelation and multiscale structure than the original variable \mathbf{x} . More details can be found in Wagner and Dray (2015), especially concerning the case of multivariate data.

2.3 | Applying the MSR procedure to test the Mantel statistic

We considered two cases: (a) if data from \mathbf{D}_X originate from a raw data table \mathbf{X} or (b) if data have been obtained directly as distances in \mathbf{D}_X . The complete procedure consists in:

1. Compute the observed Mantel statistic $r_{M\text{-obs}}$ between \mathbf{D}_X and \mathbf{D}_Y ;
2. Build a MSR replicate $\mathbf{D}_{X\text{-MSR}}$ of the original distance matrix \mathbf{D}_X :
 - i. if data from \mathbf{D}_X originate from a raw data table \mathbf{X} , MSR is applied on \mathbf{X} to produce a random replicate \mathbf{X}_{MSR} . Then, \mathbf{X}_{MSR}

is transformed in $\mathbf{D}_{X\text{-MSR}}$ using the same computation of distances applied to obtain \mathbf{D}_X from \mathbf{X} .

- ii. if data have been obtained directly as distances in \mathbf{D}_X , a principal coordinates analysis (PCO; Torgerson, 1958; Gower, 1966) is applied on \mathbf{D}_X . MSR is then performed on the complete set of principal coordinates to produce a random replicate \mathbf{X}_{MSR} . Euclidean distances are computed from \mathbf{X}_{MSR} to obtain $\mathbf{D}_{X\text{-MSR}}$.
3. Compute the Mantel statistic $r_{M\text{-MSR}}$ between $\mathbf{D}_{X\text{-MSR}}$ and \mathbf{D}_Y .
 4. Repeat n_{MSR} times the steps 2 and 3 (e.g. $n_{\text{MSR}} = 999$). The p -value of the test is then simply the number of $r_{M\text{-MSR}}$ that are higher or equal to the observed value $r_{M\text{-obs}}$ (plus one) divided by $(n_{\text{MSR}} + 1)$ in the case of an upper-tailed test.

The value of the observed Mantel statistic can eventually be corrected to take into account the spurious correlation due to spatial autocorrelation, as follows:

$$r_{M\text{-obs}}^* = r_{M\text{-obs}} - E(r_{M\text{-MSR}}) \quad (8)$$

where $E(r_{M\text{-MSR}})$ is the average of the $r_{M\text{-MSR}}$ values and corresponds to the expected value of the Mantel statistic under the null hypothesis $H_{0\text{-MSR}}$ stating that "considering the levels of spatial autocorrelation in original data, the distances in \mathbf{D}_X are not linearly related to the corresponding distances in \mathbf{D}_Y ." As such, the MSR procedure allows using a new null hypothesis compared to the Mantel test to take into account spatial autocorrelation by randomizing the original data while preserving their spatial structures. The method is schematically represented in Figure 1 and denoted MSR Mantel in the rest of the study.

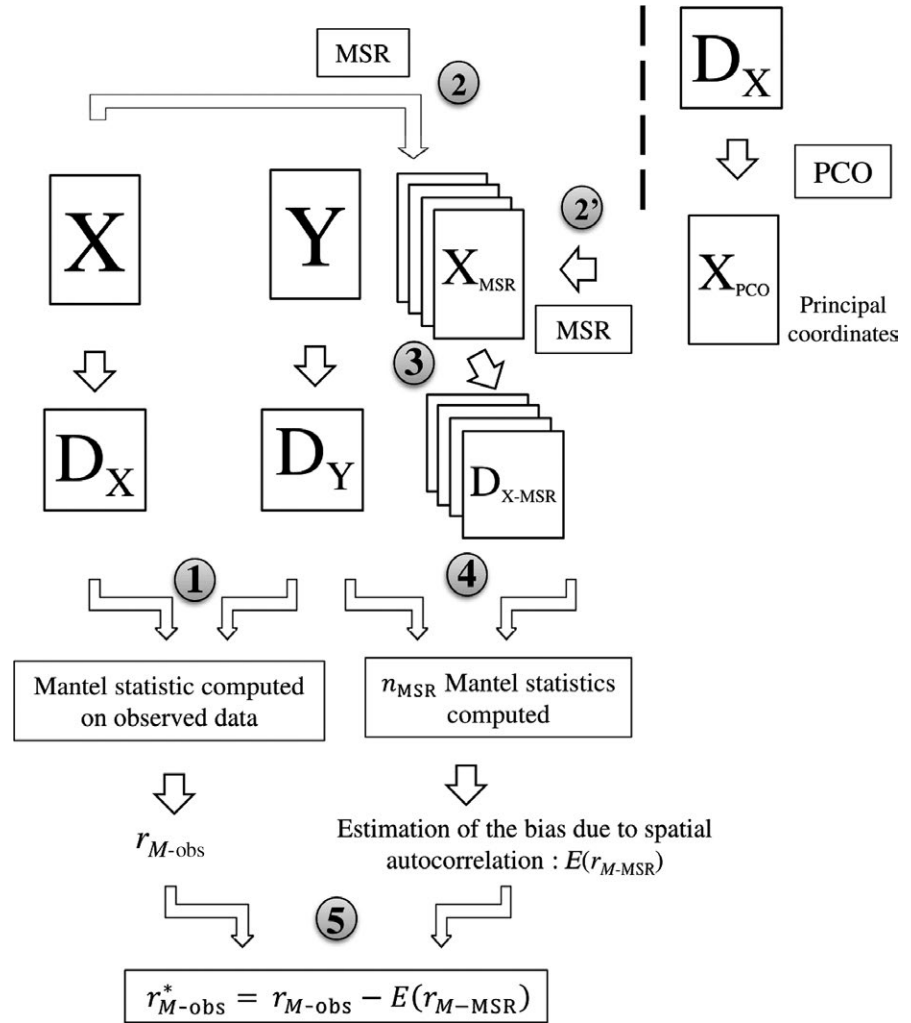
2.4 | Simulations

To assess the performance of the MSR Mantel approach to correct for the spurious correlation found in simple and partial Mantel tests, we conducted a simulation study. To evaluate type I error rates and values of the statistics under the null hypothesis, two tables \mathbf{X} and \mathbf{Y} with identical dimensions were independently generated by randomly drawing values from a normal distribution. We considered the measurement of 5 variables for 225 randomly located samples (i.e. irregular sampling design; geographic coordinates are drawn from two independent uniform distributions). Following Dray (2011), variables in \mathbf{X} and \mathbf{Y} were generated spatially autocorrelated using a univariate simultaneous autoregressive model with increasing levels of autocorrelation (autoregressive parameter ρ varying from 0 to 0.8) and a row-standardized spatial weighting matrix defined by a Gabriel graph (\mathbf{W}_{gab}).

We assessed the effect of (a) the level of spatial autocorrelation ($\rho = \{0, 0.2, 0.4, 0.6, 0.8\}$) for 5 variables and 225 samples, (b) the number of variables by considering 1, 5 and 10 variables for 225 samples in both \mathbf{X} and \mathbf{Y} with $\rho = 0.8$; (c) the number of samples by generating 100, 225 and 400 samples for 5 variables with $\rho = 0.8$ and (d) the sampling design with 225 samples located on a square grid with rook specification (i.e. regular sampling).

To evaluate power, \mathbf{X} was simulated with the same protocol as above with 5 variables, 225 irregular samples and $\rho = 0.8$. However,

FIGURE 1 Schematic representation of the MSR-Mantel procedure using Moran spectral randomization as a spatially constrained null model. (1) Distance matrices D_X and D_Y are, respectively, computed from matrices X and Y (case with raw data). The statistic r_{M-obs} is computed between distance matrices D_X and D_Y . (2) If distance matrix D_X originates from raw data matrix X , n_{MSR} replicates X_{MSR} preserving spatial autocorrelation of table X are generated with MSR. (2') If D_X does not originate from raw data, a principal coordinates analysis (PCO) is conducted on D_X and the replicates X_{MSR} are generated by applying MSR on the complete set of principal coordinates. (3) Distance matrices D_{X-MSR} are then computed from the n_{MSR} X_{MSR} replicates. (4) n_{MSR} Mantel statistics are then computed between D_Y and D_{X-MSR} to produce r_{M-MSR} . The expected value $E(r_{M-MSR})$ is an estimate of the bias due to spatial autocorrelation (i.e. expected under H_{0-MSR}). (5) The effect of spatial autocorrelation is removed from r_{M-obs} to obtain the unbiased statistic r_{M-obs}^*



Y was generated as linearly correlated with X with the following formula:

$$Y = aX + (1 - a)N \quad (9)$$

where a is a real number controlling for the strength of the link between X and Y , and N is a table of nonspatially structured random noise obtained by permuting the rows of X (this allows to ensure that both X and N have the same level of variance). We tested for the strength of the relationship between X and Y by varying values of a from 0.1 to 0.5.

To describe space (table Z) in the partial Mantel tests, we used the geographic coordinates of the sites. Distances matrices were obtained by computing Euclidean distances from tables X , Y and Z . In these simulations, the MSR procedure was performed using W_{gab} , that is, the spatial weighting matrix also used to generate the data. We performed 1,000 simulations for each scenario.

In the case of real datasets, an important step of the MSR procedure lies in the definition of the spatial weighting matrix W . Hence, in a second simulation study, we evaluated the procedure of Bauman, Drouet, Fortin, and Dray (2018) recently developed to select a spatial weighting matrix W among a set of potential candidates. In the case of MSR, this procedure consists in three main steps (see Bauman et al., 2018, for further details):

1. Perform two multivariate linear regressions of X on Moran's Eigenvectors Maps associated with positive and negative eigenvalues, respectively, for each W candidate. As such, each W candidate is characterized by two adjusted R^2 with their corresponding p -value (corrected for multiple tests).
2. Add the significant adjusted R^2 for each W candidate which is then characterized by a sum of adjusted R^2 .
3. Select the W matrix with the highest sum of adjusted R^2 .

In this study, we first reported the effects of a misspecification of W on MSR performance over 1,000 simulations. We generated X with the spatial weighting matrix W_{gab} but performed MSR Mantel with a different spatial weighting matrix W_{dist} defined as a distance-based graph. In such representation, two samples are connected solely if their geographic distance is inferior to a certain threshold, defined here as the maximum branch length of the minimum spanning tree connecting all samples (i.e. the most parsimonious path connecting all samples, see Legendre & Legendre, 1998 for details in neighbour graph definitions). Second, we evaluated the ability of the selection procedure proposed by Bauman et al. (2018) in the context of MSR Mantel. For this, X was simulated 1,000 times using

five different spatial weighting matrices (200 tables \mathbf{X} generated for each definition, see Legendre & Legendre, 1998): (a) \mathbf{W}_{gab} , (b) \mathbf{W}_{dist} , (c) \mathbf{W}_{mst} obtained from a minimum spanning tree, (d) \mathbf{W}_{del} defined with a Delaunay triangulation and (e) \mathbf{W}_{rel} obtained from a relative neighbourhood graph. We applied the selection procedure considering all the five spatial weighting matrices as candidates and evaluate type I error rates of the MSR Mantel conducted using the selected spatial weighting matrix. The study on misspecification and selection procedure considered \mathbf{X} and \mathbf{Y} generated as previously with an autoregressive model of parameter $\rho = 0.8$ and with 5 variables and 225 irregular samples.

2.5 | Statistical analysis

For each pair of distance matrices, we applied simple and partial Mantel tests, and the MSR-Mantel procedure. Using the 1,000 simulations, we computed type I error rates corresponding to the proportion of significant relationships identified when \mathbf{X} and \mathbf{Y} are not linked (i.e. false positives). In the cases where \mathbf{X} and \mathbf{Y} are linearly correlated, the proportion of significant relationships represents the power of the test. We used 999 permutations for the simple and partial Mantel tests, and 99 replicates for the MSR procedure to reduce the computation time. Statistical tests and simulations were

computed with R software 3.3.2 (R Core Team, 2016). Simple and partial Mantel tests were, respectively, computed with ADE4 (Dray & Dufour, 2007) and VEGAN (Oksanen et al., 2017) packages. MSR procedure was performed using ADESPATIAL package (Dray et al., 2016). Examples showing how to reproduce the analysis and the selection procedure in R are provided in Supporting Information Appendix 5.

3 | RESULTS

When $\mathbf{D}_\mathbf{X}$ and $\mathbf{D}_\mathbf{Y}$ were not correlated (i.e. \mathbf{X} and \mathbf{Y} independently generated), $r_{M\text{-obs}}$ was expected to be 0. Simple Mantel test performed well when there was no spatial autocorrelation (the $r_{M\text{-obs}}$ are centred on 0 and type I error rate close to 0.05 for $\rho = 0$; Figure 2a). On the contrary, type I error rates and $r_{M\text{-obs}}$ were increasingly inflated with higher levels of spatial autocorrelation (Figure 2a) and this bias increased with the number of variables (Figure 3a) but decreased with the number of samples (Supporting Information Appendix 1a). Note also that the variance of $r_{M\text{-obs}}$ on 1,000 simulations increased with the level of spatial autocorrelation (Figure 2a). Likewise, type I error rates and $r_{M\text{-obs}}$ presented similar inflations for a regular sampling design (Supporting Information Appendix 2a, 3a and 4a). Worth noting that in the case of regular sampling grids $r_{M\text{-obs}}$ was

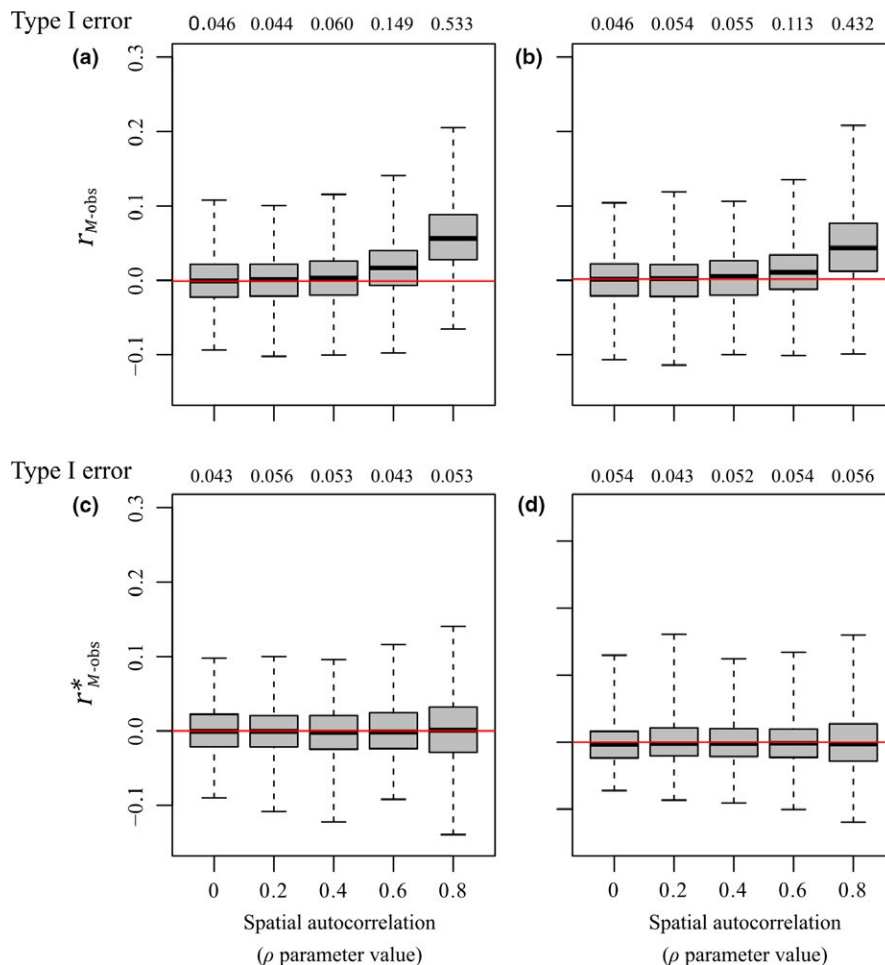


FIGURE 2 Type I error rates and values of Mantel statistic for simple and partial Mantel tests and MSR Mantel for irregular sampling. (a–b) Observed Mantel statistic ($r_{M\text{-obs}}$) and type I error rates for (a) simple and (b) partial Mantel test. (c–d) Corrected Mantel statistic ($r_{M\text{-obs}}^*$) and type I error rates of MSR Mantel on (c) raw table \mathbf{X} , or (d) the principal coordinates obtained from a (PCO) on $\mathbf{D}_\mathbf{X}$. \mathbf{X} and \mathbf{Y} contain 5 variables and 225 samples. Both tables present spatial autocorrelation varying from $\rho = 0$ to $\rho = 0.8$. $r_{M\text{-obs}}$ and $r_{M\text{-obs}}^*$ are reported with boxes representing the 25, 50 and 75 quartiles, the whiskers are minimum and maximum values. Red line corresponds to $r_M = 0$

not overestimated and type I error rate presented no inflation for the lowest levels of spatial autocorrelation (Supporting Information Appendix 2a).

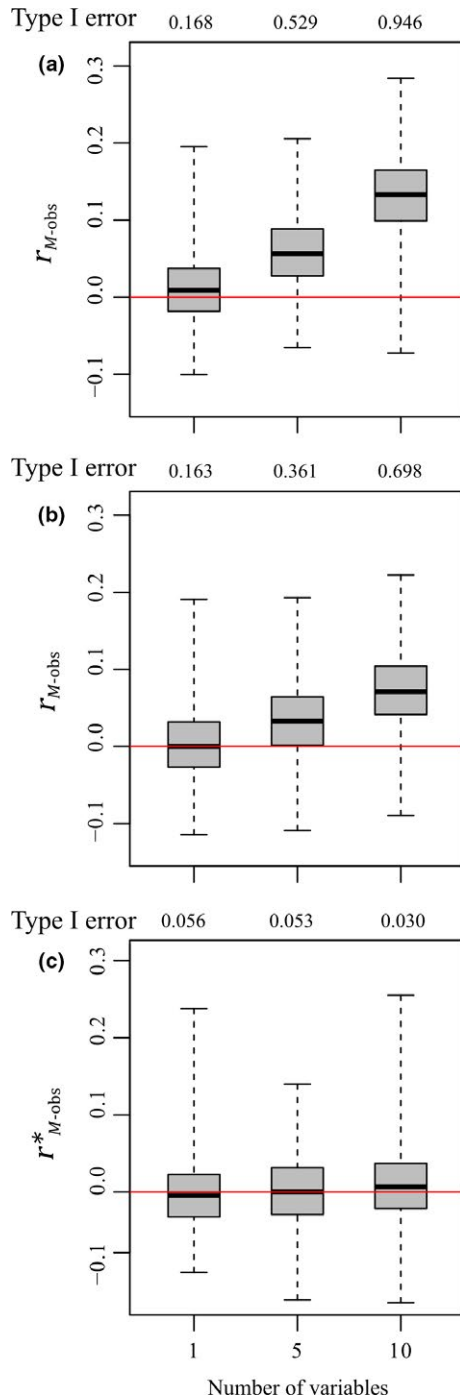


FIGURE 3 Type I error rates and values of Mantel statistic for simple and partial Mantel tests and MSR Mantel for irregular sampling with increasing number of variables. (a–b) Observed Mantel statistic (r_{M-obs}) and type I error rates for (a) simple and (b) partial Mantel test. (c) Corrected Mantel statistic (r^*_{M-obs}) and type I error rates of MSR Mantel on raw table X. (a–c) X and Y contain 225 samples and increasing number of variables: 1, 5 and 10. Both tables present spatial autocorrelation fixed to $\rho = 0.8$. r_{M-obs} and r^*_{M-obs} are reported as in Figure 2. Red line corresponds to $r_{M=0}$

As observed in other studies, partial Mantel tests did not control for the spatial autocorrelation effect: the r_{M-obs} statistic remained overestimated and, although improved, type I error rates were still inflated (Figure 2b). As previously observed for the simple Mantel tests, the number of variables increased r_{M-obs} and type I error rates (Figure 3b), and inversely for the number of samples (Supporting Information Appendix 1b). However, worth noting that in the case of regular sampling grids, even if r_{M-obs} was still overestimated, type I error rates presented lower inflations (Supporting Information Appendix 2b, 3b and 4b).

The use of the MSR Mantel fully controlled for inflations due to spatial autocorrelations so that estimates and type I error rates behave as expected (r^*_{M-obs} and type I error rates, respectively, centred on 0 and 0.05; Figure 2c). Similarly, the procedure succeeded for the various situations considered: increasing number of variables (Figure 3c), increasing number of samples (Supporting Information Appendix 1c) and for regular sampling (Supporting Information Appendix 2c). Moreover, even when conducted on the principal coordinates of D_X and not on raw data, the use of MSR procedure successfully controlled for spatial autocorrelation biases (Figure 2d). Note, however, that our approach did not correct the increase in the variance for high level of spatial autocorrelation (Figure 2c, Supporting Information Appendix 2c).

When D_X and D_Y were linearly correlated, the performances of the Mantel tests (simple, partial) and MSR Mantel were very similar and their power increased with the strength of the correlation between the two matrices (Figure 4). These similarities showed that MSR Mantel did not affect the ability to detect a link between D_X and D_Y when present. However, as simple and partial Mantel tests had inflated type I error rates (Figure 2a,b), they should not be used and their power was only given for comparison purposes.

Performing MSR Mantel with a misspecified spatial weighting matrix (W_{dist} instead of W_{gab}) led to an inflated type I error rate (0.115 over 1,000 simulations). When the selection procedure developed by Bauman et al. (2018) is applied prior to MSR Mantel, the type I error rate drops down to 0.049.

4 | DISCUSSION

In this study, we developed a new procedure based on MSR (Wagner & Dray, 2015) to overcome the biases when testing the Mantel statistic when distance matrices D_X and D_Y present independent spatial autocorrelations.

As shown previously (Guillot & Rousset, 2013), we found that simple Mantel tests performed well in the absence of spatial autocorrelation but that the statistic and associated type I error rates were spuriously inflated as soon as spatial autocorrelation was introduced. Moreover, these inflations increased with the number of variables. This trend was expected as a higher number of independent spatially structured variables in both distance matrices leads to a higher diversity of spatial patterns and thus higher chances to obtain spurious correlations between D_X and D_Y . On the contrary,

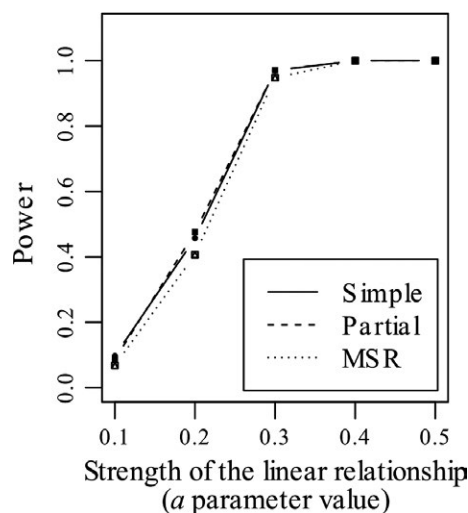


FIGURE 4 Power of simple and partial Mantel tests and MSR Mantel for irregular sampling when X and Y are linearly related. Comparison of the power of simple (solid line) Mantel test, partial (dashed line) Mantel test and MSR Mantel (dotted line). X and Y contain 225 samples and 5 variables. Both tables present spatial autocorrelation fixed to $\rho = 0.8$. The strength of the linear relationship varied from $a = 0.1$ to $a = 0.5$

increasing the number of samples reduces the effects of spatial autocorrelation, as such decreasing the detection of spurious correlations. Likewise, partial Mantel tests presented similar inflations of type I error rates and estimations with the same trends relative to the number of variables and samples. However, partial Mantel test biases were lower than for simple Mantel test due to the usage of the geographic distance matrix D_z to consider space. Worth noting that for high spatial autocorrelation ($\rho = 0.8$), the regular sampling offered a better Type I error even in the case of high number of variables and low number of samples. To sum up, as reported by Guillot and Rousset (2013), we confirmed that partial Mantel tests failed to adequately correct for the effect of spatial autocorrelation observed in Mantel tests.

In contrast, our approach based on MSR procedure provided acceptable levels for type I error rates when distance matrices were independently generated but both spatially autocorrelated. On the other hand, when distances matrices were linearly correlated and spatially structured, our procedure detected the relationship with a high statistical power. This demonstrates the efficiency of our procedure to correct for the spurious correlation induced by spatial autocorrelation, while conserving the ability to detect correlations when present. In addition, our procedure can be applied on regular as well as irregular samplings, commonly used in ecological surveys (e.g. Saito, Soininen, Fonseca-Gessner, & Siqueira, 2015; Tuomisto et al., 2016). Besides, by subtracting the expected value of the Mantel statistic under H_{0-MSR} , our formula provided a correction to the Mantel statistic but does not improve its precision as the variance of the statistic is not transformed. As such, computing standardized effect size (SES; e.g. Gotelli & McCabe, 2002) by dividing Equation (8) by the standard deviation of MSR replicates would

probably be more adapted to compare the values of the corrected Mantel statistics between studies.

While this new procedure is promising, it has some limitations. MSR Mantel relies on MSR whose first step is to define a spatial weighting matrix W . The specification of W plays an important role in determining the appropriate form of spatial model (e.g. Stakhovych & Bijmolt, 2009 in the case of spatial autoregressive models). Hence, its misspecification can greatly influence the performance of our procedure by defining incorrectly the potential spatial dependence between observations. Indeed, we showed that, when W is misspecified, MSR Mantel failed to control for the inflation of type I error rates in the presence of spatial autocorrelation. Our results indicate that the selection procedure proposed by Bauman et al. (2018) offers a promising solution to optimize the choice of W among a set of candidates. Furthermore, the MSR procedure is only able to deal with continuous variables in X , excluding counts, binary and categorical variables. Moreover, in the case where data have been obtained directly as distances matrices, our procedure based on principal coordinates analysis assumes that data can be represented in a Euclidean space. Hence, further work is required to extend these promising results to other types of variables and non-Euclidean distance matrices. When non-Gaussian response variables are expected, alternative methods based on generalized linear mixed models may be considered. In genetics, for instance, Guillot, Vitalis, le Rouzic, and Gautier (2014) developed a spatially explicit model that directly consider autocorrelation and Rousset and Ferdy (2014) presented fitting procedures for spatial GLMM providing correct estimate of correlation parameters. However, spatial GLMM are only suitable when raw data are available or when they can be reconstructed from available distance matrices and, as the MSR Mantel, they can be sensitive to the specification of the spatial model (Duncan, White, & Mengersen, 2017).

Our approach aims to consider spatial autocorrelation when studying the link between two distance matrices. From a theoretical viewpoint, this issue pertains to the necessity to account for nuisance parameters during the analysis of parameters of interest. Raufaste and Rousset (2001) designed a simple simulation model where the objective is to study the effect of an environmental variable on the abundance of a species at location $k(x_k)$ in the presence of migration flows from the two adjacent populations (x_{k+1} and x_{k-1}). They showed that “the partial Mantel test is inadequate in this model because the permutations will not hold constant the (minimal) sufficient statistic for the nuisance parameter under the null hypothesis.” In their model, these statistics are $(\sum_k x_k, \sum_k x_k^2, \sum_k (x_{k+1} + x_{k-1}) x_k, \sum_k (x_{k+2} + x_{k-2}) x_k)$ and the MSR procedure, by preserving the mean, variance and global level of autocorrelation measured by Moran's index (Wagner & Dray, 2015), holds constant the first three elements, but not the fourth. However, results showed that type I error rates were controlled in all cases with our simulation design suggesting that the MSR-Mantel procedure seemed quite robust. An alternative is to use the MSR-Mantel procedure in the context of maximized Monte-Carlo (Dufour, 2006) so that the distribution of the statistic under the null hypothesis

is built for the values of sufficient statistics that maximized the *p*-value. This ensures that the test is exact.

In conclusion, our results confirmed Guillot and Rousset (2013) findings and suggest that several studies ranging from genetic (e.g. Shafer & Wolf, 2013) to community ecology (e.g. Astorga et al., 2012) could have wrongly identified an effect when standard or partial Mantel tests were used in the presence of spatial autocorrelation. Spatial autocorrelation is a problem regularly underlined when quantifying the spatial structure of genetic (Manel et al., 2010) and community data (Gilbert & Bennett, 2010; Smith & Lundholm, 2010) and our procedure could solve this issue by providing an alternative distance-based statistical approach.

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AUTHORS' CONTRIBUTIONS

S.D. and T.D. devised and helped supervise the project; S.D. developed the theory; J.C. and S.C. performed the computations, analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

This study does not include any data.

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